Dalton (J. C.)

ON THE

SPECTRUM OF BILE.

A PAPER READ BEFORE THE NEW YORK ACADEMY OF MEDICINE, JANUARY 8, 1874.

BY

JOHN C. DALTON, M. D.,

PROFESSOR OF PHYSIOLOGY IN THE COLLEGE OF PHYSICIANS AND SURGEONS.

Presented by the Author

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ON THE SPECTRUM OF BILE.

The nature and properties of the coloring matters of bile are not yet fully understood. The secretion varies considerably in hue in different species of animals, and even in different specimens from the same animal, owing apparently to its different degrees of concentration and to the presence, in varying proportions, of different ingredients. In various specimens of normal bile we may meet with all the intermediate tints of golden-yellow, yellowish-red, reddish-brown, olive-brown, olive, yellowish-green, and pure green. Human bile is generally of a dark golden-brown; pig's bile of a reddish-orange or reddish-brown; dog's bile of a brownish-olive or bronze color; and sheep and ox bile of a greenish-olive, or more frequently of a pure green. All these differences may be referred to two main classes of tints, in one of which the predominating color is red or reddish-brown, while in the other it is green.

So far as chemical investigation has yet gone, it seems also that two principal biliary coloring matters have been more or less distinctly recognized. One of them is *bilirubine*, a nitrogeneous crystallizable matter, of a reddish-yellow color; the other *biliverdine*, also nitrogeneous and imperfectly crystallizable, which is green. A solution of the reddish-yellow bilirubine becomes changed into the green biliverdine by exposure in thin layers to the oxygen of the air; and a specimen of bile which has originally only a yellowish or olive

color may also be turned green by exposure to the air, or by other oxidizing agents, such as nitric acid, or a watery solution of iodine.

Beside these two coloring matters, several others have been enumerated by various authors; but their characters are not very well defined, and there appears to be hardly sufficient evidence that they are really normal ingredients of the secretion.

In the present paper I propose to speak of two different subjects: First, the spectrum presented by fresh bile, which depends on the presence of its normal coloring matters; and, secondly, the spectrum presented by the colored fluid of Pettenkofer's test, which depends for its production on the presence of the biliary salts.

I. The spectroscopic characters of the bile are by no means agreed upon. By several observers either one or two absorption bands are given at varying points between the spectrum-lines D and E. On the other hand, it is said by some that fresh bile produces no distinct modification of the spectrum, and Vierordt remarks, in a recent publication,[1] that the variety and changeability of its coloring matters are such as to preclude any definite description of their effects without further examination.

Nevertheless I believe, from my own observations, that the bile presents a very distinct and characteristic spectrum. It is not, perhaps, so well marked as that of the blood, but it is still sufficiently so to be important and useful as a means of determining the ingredients of the secretion.

I have examined for this purpose forty-five different specimens of bile, namely, thirteen specimens from the ox; nine from the sheep; eleven from the pig; eight from the dog, and four specimens of human bile. With the exception of the human bile, and, in one case, of dog's bile, all these were taken from the body immediately after death, and either examined the same day or kept in a cool place to be filtered or to settle clear till the next day, in order to preclude any alteration of their natural ingredients.

[1] "Die Anwendung des Spectral-Apparates zur Photometrie der Absorptions-Spectren." Tübingen, 1873, p. 140.

Ox-bile and sheep's bile is often clear when taken out of the gall-bladder, or, if not so, is easily filtered. Pig's bile is usually quite turbid, but, with the use of some care, may be filtered clear through ordinary filtering-paper. Dog's bile, as a general rule, will not pass through filtering-paper, but may be rendered perfectly clear by being allowed to stand for some hours in a narrow, cylindrical, upright vessel, the impurities subsiding to the bottom. The specimens may then be examined by placing them before the slit of the spectroscope in layers of one, two, three, or four centimetres' thickness.

The mode of indicating the measurements of the spectrum which I have found most useful for physiological purposes is that adopted by Vierordt. In this method we take, to begin with, as fixed points, the eight principal lines of the solar spectrum, A, B, C, D, E, F, G and H. The spaces between these lines are then considered as divided, each into one hundred equal parts; and the situation of an absorption-band is expressed by proportional numbers, counting in a direction from the red toward the violet end of the spectrum. Thus, if a narrow band, or the commencement of a wide one, be placed midway between F and G, its situation is expressed by the formula, F 50 G. If it be placed at only one-quarter the distance, counting from F to G, it is said to be at F 25 G; if at three-quarters the distance, it is at F 75 G. This plan allows a much greater accuracy of description than the indefinite expressions frequently employed, and is at the same time sufficiently exact for all measurements needed in physiological examinations.

The first distinguishing character of the spectrum of bile is that it is *very short*, the light being totally absorbed at a considerable distance from the refrangible end. In the specimens of ox-bile, viewed in a thickness of one centimetre, in no case did the spectrum extend beyond the line F in the first quarter of the blue, and in most instances fell considerably short of it. The average limit of the spectrum was toward the end of the green, at E 60 F. In sheep's bile, viewed in a thickness of one centimetre, the spectrum also terminated within the line F, except in one case, where it extended just beyond that point. Its average limit was in the latter part of the green, at E 45 F.

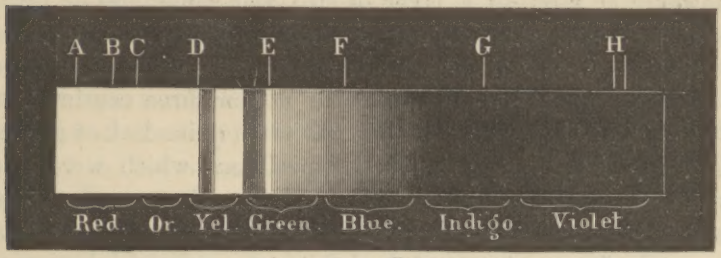
In the reddish-brown, olive, and yellowish-brown bile of the dog, the pig, and the human subject, the spectrum was shorter still; terminating, in pig's bile, on the average, in the first half of the green at the line E, in dog's bile just beyond this point, and in human bile, also in a thickness of one centimetre, within this limit, near the commencement of the green, at D 82 E. When viewed in layers of two centimetres' thickness, the spectrum was proportionately shortened; terminating, on the average, in ox-bile at E 33 F, in pig's bile at D 85 E, and in dog's bile at D 75 E. The bile will even bear a considerable degree of dilution without losing this peculiarity. A specimen of human bile, of a golden-brown color, which, in a layer of one centimetre, gave a spectrum ending a little before E, when diluted with thirty volumes of water was nearly colorless to the eye in a layer of one centimetre, but its spectrum did not extend beyond F 28 G. A specimen of reddish-orange pig's bile, the spectrum of which terminated just beyond the line E, when diluted with fifty volumes of water, still gave a spectrum that fell a little short of the line G.

As a general rule, therefore, the absorptive power of the bile is remarkably strong for the more refrangible rays, and its visible spectrum is accordingly very short. This character is exhibited by the spectrum of all kinds of bile, but it is rather more marked in the ruddy and yellowish-brown kinds than in specimens where the predominating color is green.

In the second place, the spectrum of bile does not fade away gradually like that of many other colored fluids, but *terminates suddenly.* This is a very well-marked character, and, in the thirty-five observations in which attention was given to this point, it was present in all but two, in sufficient distinctness to constitute a striking feature of the bile-spectrum. In the spectrum of blood, the fading of the light toward the refrangible end is very gradual, even when the two characteristic absorption-bands are well marked. Defibrinated dog's blood, diluted with one hundred parts of water, and viewed in a thickness of one centimetre, gives both the absorption-bands very strongly pronounced; and at the same time there is a progressive dimness throughout the latter portion of the

green and the whole of the blue, the visible spectrum terminating entirely about F 50 G. But, in the spectrum of bile, this gradual fading is absent, and the light is cut off suddenly, making a strong contrast with the complete darkness immediately beyond its limit. This appearance is perceptible in bile of all shades of green, olive, yellow, and reddish-brown.

FIG. 1.



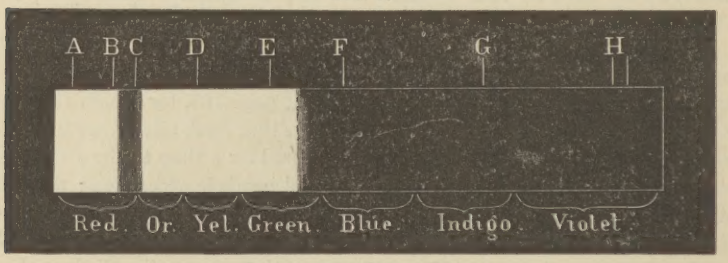
SPECTRUM OF BLOOD.

The third peculiarity of the bile-spectrum is the presence of an *absorption-band in the red*, at the situation of the line C. So far as I am aware, this band has not heretofore been noticed by any of the writers on the subject.[1] And yet, ac-

[1] Since this paper was presented to the Academy, I have met with some observations of Bogomoloff, cited by Fumouze in his work, "Les Spectres d'Absorption du Sang," Paris, 1871, page 135, for which I am indebted to Dr. H. G. Piffard. Bogomoloff says that fresh bile presents no absorption-spectra; but that, after being exposed for a time to the air, it becomes acid in reaction, and then shows several bands in succession; the first one appearing to the right of the line D; the second, "after one or two days," to the left of D; later still, *a third at C;* and finally, a fourth at the situation of the line E, the last two being always less distinct than the two others. I have so often seen the band at C very strongly pronounced, in fresh bile, without any other band being visible at the time, that I can hardly think the observations of Bogomoloff and my own refer to the same thing. The appearance in question is certainly not dependent on an acid reaction or any want of freshness of the bile. I have found the C band perfectly distinct in neutral and alkaline sheep's bile, which was examined within an hour, three-quarters of an hour, thirty-seven minutes, half an hour, and in one case within fifteen minutes after the animal was killed and the gall-bladder taken out of the abdomen. In these cases the bile was kept during the interval in the gall-bladder, and was taken out only at the moment of subjecting it to spectroscopic examination; so that there can be no reasonable doubt that this band is a spectroscopic feature of the normal bile, and not a consequence of its change or decomposition.

cording to my own observations, it is so constant and so well marked as to form a characteristic feature in the spectrum of bile whenever it has a decided greenish tint, and often when it is of a yellowish, reddish, or olive-brown color. In eleven specimens of ox-bile, sheep's bile, and dog's bile, of a green, greenish-olive, olive, or olive-brown color, this band was visible when examined in a thickness of only one centimetre. Usually, however, it requires a layer or two, and sometimes of three centimetres, to bring it out distinctly. In all the nineteen specimens of ox and sheep's bile which had a greenish or olive tint, when viewed in a thickness of two or three centimetres, the band at C was very distinct and often quite dark or almost black. In the three remaining specimens, which were of a yellowish-brown color, without any tinge of green, the band was but faintly visible in layers of three or even four centimetres. In five specimens of dog's bile, of a yellowish-brown or olive-brown color, it was distinct and sometimes very dark in a layer of two centimetres; and in a sixth it was perceptible in a layer of three centimetres.

Fig. 2.



Spectrum of Green Bile.

In these cases, as a general rule, the intensity of the band at C is in proportion to the preponderance of green in the color of the bile. Though easily seen, in comparatively thin layers, in specimens of a pure green or a decided greenish-olive color, it is less perceptible in specimens of a yellowish, yellowish-brown, or olive-brown tint. But if a specimen of reddish or yellowish-brown bile, which does not show the band distinctly, be treated with a few drops of iodine solution until it turns of a decided green, the band at C immediately becomes visible, often in a very marked manner. The same

effect is produced by exposing the bile to the air until it assumes a green color. I have observed in this way the appearance or increased intensity of the C band, under treatment with iodine or atmospheric exposure, in nine specimens of ox, sheep, and dog's bile.

It would appear from this result that the C band is probably due to the presence of the green rather than the red coloring matter of the bile. Since the red coloring matter, or bilirubine, is well known to be converted into biliverdine by oxidizing agents, and as this change of color is accompanied by the appearance of the C band, it is difficult to avoid the conclusion that the two are directly connected with each other. At the same time, I have seen the band very dark and distinct on one occasion, in a thickness of one centimetre, in dog's bile which was of a deep olive-brown color; and twice it was faintly visible, in two and three centimetres in specimens which were brownish-yellow. In both the latter cases, the band became dark and distinct after the bile was turned green by iodine.

The band also disappears from the spectrum of ox-bile when this fluid loses its green tint. If ox-bile, of a pure green or olive-green color, which shows a distinct band at C, be inclosed in a perfectly full and securely stopped vessel, so as to be entirely protected from the air, it gradually loses its color; and at the end of twelve, twenty-four, or thirty-six hours, has become of a light-yellow, or yellowish-brown, without any remaining tinge of green. If examined in this condition, the band at C is no longer visible; and in a series of specimens it becomes indistinct exactly in proportion to the fading away of the green color. After it has completely disappeared, it may be at once restored by again turning the bile green with a solution of iodine.

This change appears to be analogous to that which takes place in blood, when the characteristic absorption-bands of aërated blood disappear after the fluid has been kept for two or three days in a tightly-stopped bottle, and are at once restored by shaking it up with oxygen or atmospheric air. The green tint of the biliary coloring matter, at least in ox-bile and similar varieties, seems to be dependent upon continued

oxidation, and to be indicated in this condition by a special absorption-band.

The usual position of the band is at the line C, but extending to a greater distance on its left than on its right side; namely, from B 20 C to C 10 D. In one case, where it was very strong and dark, it extended from B 13 C to C 18 D; and in one or two instances the absorption began at or just beside the line B, thus occupying the entire space between B and C. Its extreme limit, in the opposite direction, was in one case where it extended, in a thickness of three centimetres, from B 30 C to C 25 D. It invariably included the line C, and never passed beyond either of the adjacent lines.

In the eleven specimens of pig's bile which were all of a reddish-orange, yellowish-brown, or ruddy-brown color, I did not find the C band indicated, even in a thickness of three centimetres. In six of the specimens, treatment with iodine produced a green or olive color, after which the band became visible, though usually quite faint. In the remaining five cases no green or greenish color was developed by the addition of iodine; and there was no appearance of the band at C.

It seems probable therefore, that in pig's bile the principal coloring matters may be different from those in the bile of the other domesticated animals.

With regard to other absorption-bands in the bile, I have seen, in three instances of olive-green, yellowish-brown, and red bile, a very narrow and faint band at D 18 E. There is reason to believe that this was really one of the two absorption-bands of blood, with the first of which it corresponds precisely in situation; the second blood-band being imperceptible owing to its comparative faintness and the termination of the spectrum within or just beyond its limits. In two instances I have met with both blood-bands in the spectrum of bile. One was the case of a woman, who had died of nephritis; the gall-bladder being taken out at the autopsy next day. The filtered bile was of a ruby-red color, and in a layer of one centimetre showed both the absorption-bands of blood, with their normal relations to each other; one narrow and distinct, just beyond D, the other wider and fainter, from

D 60 E to E. The last band, however, was not isolated, because the spectrum itself, as not unusual with bile, terminated suddenly at E, thus leaving the band only in the form of a distinct dimness of the latter part of the spectrum.

In the case of a dog, where the gall-bladder was left in the abdomen for several hours after death, the bile also contained blood. It was of a clear claret-red color, and showed both the absorption-bands distinct and isolated. The spectrum ended suddenly, some distance beyond the second band, at E 47 F.

I have found that a similar spectrum may be produced by adding defibrinated blood to fresh dog's bile, in the proportion of one drop of blood to three cubic centimetres of bile. Furthermore, if blood be diluted by successive additions of water, so as to weaken its spectrum, the first absorption-band always remains visible longer than the second. If the dilution be carried to the extent of one part of blood to one thousand parts of water, and the mixture viewed in a thickness of one centimetre, the first band is still visible, though faint, while the second is entirely imperceptible. In the spectrum of bile, therefore, the occasional faint band at D 18 E is no doubt the first absorption-band of blood, which has been exuded into the gall-bladder during the last hours of life or within a short time after death.

There are also two other bands sometimes to be seen in the spectrum of bile, one situated at D, the other at D 30 E. The first is usually quite imperceptible as a band, existing only in the form of a more or less diffused dimness in the region of the yellow; but sometimes, in an instrument which gives a very short and compressed spectrum, like the Sorby-Browning micro-spectroscope, it shows itself as a dark striation at this point. It may appear in either greenish or yellowish-brown bile. The other absorption-band, namely, that at D 30 E, I have seen, accompanying the C band, in ten specimens of ox and sheep's bile, which nearly all had a greenish or olive tinge.[1] This band, however, like the preceding, is uncertain in its appearance, always quite faint, and often nothing more than a slight indication of the absorption

[1] This is probably the first band mentioned by Bogomoloff as appearing in bile during its alteration by exposure to the air.

of light in that region. It is never to be compared, for intensity or distinctness, with the band at C, and in my own observations was entirely absent in a considerable portion of the cases examined.

The band at C, accordingly, is the only one which is sufficiently constant and distinct to be regarded practically as a characteristic feature of the spectrum of bile.

Finally, the spectrum of bile, as a general rule, exhibits a remarkable *diminution in quality of the orange and yellow colors.* In the great majority of cases there is but little orange perceptible, and no pure yellow. The place of the orange is occupied by red, which extends farther toward the right than in the normal spectrum; while, in the opposite direction, the yellow is encroached upon by the green. Between these two, in the situation of the pure yellow, there is a perceptible diffused dimness; and it is this dimness which sometimes assumes the appearance of a faint absorption-band at the line D. Very often, however, even when there is no distinct band at this point, the pure yellow is entirely wanting; and in not a few cases the orange is also deficient, while the spectrum terminates as usual before the commencement of the blue, so that the only colors really visible in the spectrum are red and green. Even the green has a bluish tint, in comparison with that of the normal spectrum. This peculiarity in the spectrum of bile shows itself, whether the color of the specimen be greenish or yellowish-brown.

II. The well-known reaction of Pettenkofer's test for the biliary salts, which has now been in use for thirty years, is one of the most striking and elegant of all those employed in physiological chemistry. If the bile, or a solution of either glycocholate or taurocholate of soda, be treated with a small quantity of sugar and pure sulphuric acid in excess, a violet-red color is produced which is very distinctive. As the fresh bile, however, contains other organic matters which are liable to interfere with the purity of the reaction, it is indispensable, in delicate examinations, first to evaporate the bile to dryness, extract the dry residue with absolute alcohol, and then to precipitate the alcoholic solution with ether in excess—when

the biliary salts separate from the solution and soon take the form of an abundant crystallization.

A clear and colorless watery solution of the biliary salts, thus purified, is used for the test. Only a small quantity of sugar is added, because if it were abundant a discoloration might be produced by its own reaction with the sulphuric acid. One part of cane sugar, dissolved in four parts of water, makes a convenient solution for ordinary use. Of this saccharine liquid, one drop is added to each cubic centimetre of the solution of biliary salts. This produces at first no visible change. On adding a few drops of sulphuric acid, the acids of the biliary salts are decomposed, and cholic acid is precipitated, forming in the solution a white cloudiness. This is redissolved on increased addition of sulphuric acid, the liquid becoming clear, with the development of minute bubbles of gas. Soon afterward there appears a pink or cherry-red color, which gradually deepens into a ruby-red, a violet, and finally, if the biliary salts were abundant, a deep opaque purple. If the solution be very dilute, the violet color, which is the essential feature of the reaction, may not be fully developed under five or six hours.

The value of this reaction has been thought to be impaired from the fact that a similar tint may be produced by Pettenkofer's test with certain other substances besides the biliary salts. Oleine, oleic acid, ethereal oil, amyl-alcohol, albuminous matters, and more recently[1] morphine and codeine, have all been mentioned as possessing this property. Of these substances, however, the only ones which give a reaction liable to be mistaken for that of the biliary salts are albumen and the opium alkaloids. Serum of blood, treated with Pettenkofer's test, gives a ruby-red fluid which soon acquires a violet tint, and in the course of twenty-four hours becomes a deep purple. Even when diluted with ten times its volume of water, it will give, in half an hour after the application of the test, a bright, clear, ruby-red fluid. Pure white of egg, treated in the same way, at once shows a clear ruddy

[1] Poggendorf's "Annalen," 1872, No. 9, page 128.

color, and in the course of an hour acquires a distinct violet tinge.

Both codeine and morphine exhibit a similar reaction in a solution of ten or more parts per thousand. On the addition of sugar and sulphuric acid, a nearly clear pink color, with more or less of a violet shade, is rapidly produced, which soon becomes a strong cherry-red, and, after some hours, a purple-red. The similarity of colors is most striking in the case of codeine, which may produce at the end of twelve hours a deep purple fluid, entirely undistinguishable from that caused by the ingredients of the bile.

These facts, however, do not altogether invalidate Pettenkofer's reaction as a test for the biliary salts. They would do so, if we applied the test directly to the bile or to animal fluids supposed to contain it. But this is never done. If the fluid be first evaporated, extracted with alcohol, precipitated with ether, and the precipitate dissolved in water before applying the test, most of the above substances will be excluded. Codeine is soluble in ether, and is not precipitated by it from its solution in alcohol. Albuminous matters could not be extracted by absolute-alcohol from the dry residue, and neither oleine, oleic acid, amyl-alcohol, nor ethereal oil, could be precipitated by ether from the alcoholic solution, nor dissolved in water afterward. So far as these substances are concerned, no mistake can arise if the test be thoroughly carried out.

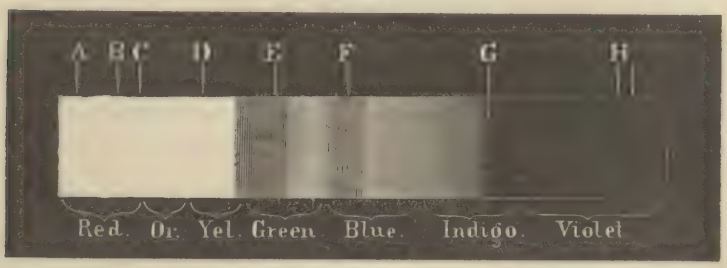
The salts of morphine, however, so far as I am aware, present an insuperable difficulty. They are soluble in water and in alcohol, and may be precipitated by ether from an alcoholic solution, very much in the same way as the biliary salts. After this precipitation, they may be dissolved in water, and will then give a red color with sugar and sulphuric acid. The coincidence is still more striking from the fact that the colored fluid thus produced, by both morphine and codeine, gives a *spectrum* so similar to that of the biliary salts that no certain distinction can be made between them, except that in the spectrum from biliary salts the peculiar characters are much more strongly marked than in that from morphine and codeine. Morphine, however, is of course not liable to be present in purely physiological investigations upon the fluids of ani-

mals. Albuminous matters are the only ones occurring as ingredients of the body which could possibly give rise to error; and these, as we have seen above, are excluded by the usual preliminaries of the test.

The spectrum of Pettenkofer's test is interesting, as affording an additional means of identifying it in certain cases, should doubt arise. Dr. S. L. Schenk,[1] of the Physiological Institute at Vienna, has published, within the past year, some observations on this subject. He operated with alcoholic solutions of pure glycocholate and taurocholate of soda, obtained from Merck's laboratory in Darmstadt, and found that these substances, treated by Pettenkofer's test, yielded a spectrum with two characteristic absorption-bands, situated, the one at E, the other at F.

I have also found these bands to exist in the spectrum of Pettenkofer's test, when carried out in the manner adopted by Dr. Schenk. I obtained the pure sodium glycocholate and taurocholate from Merck's laboratory, and applied Pettenkofer's test to these salts in alcoholic solution. The sodium glycocholate, dissolved in alcohol in the proportion of

Fig. 3.



Spectrum of Pettenkofer's Test with the Biliary Salts in Alcoholic Solution.

100 milligrammes to the cubic centimetre, gives, with sugar and sulphuric acid, a deep opaque purple fluid, which, in a suitable grade of dilution, shows in the spectroscope two wide and dark absorption-bands: one at E, extending from D 50 E to E 25 F: the other at F, from E 60 F to F 15 G, the spectrum terminating gradually about the line G. The sodium taurocholate, under similar circumstances, forms a deep

[1] "Anatomisch-physiologische Untersuchungen," Wien, 1873.

violet-red fluid, and gives a spectrum with the same two bands at E and F, in exactly the same situations and with the same characters as in the former case.

There is also frequently to be seen a band at D, which is, however, narrow and faint, and not at all to be compared for distinctness with the two others. This band is mentioned by Dr. Schenk as having been seen by Bogomoloff, though he himself did not find it. It certainly exists in many cases, but it is by no means constant.

If dried ox-bile be extracted with absolute alcohol, the alcoholic solution precipitated with ether and allowed to stand until crystallization takes place, the mixture of ether poured off, and the crystalline deposit then dissolved in alcohol and treated with Pettenkofer's test, this fluid also has a spectrum in which the E and F bands are visible, and often, also, the band at D. They vary somewhat in different specimens, the E band being usually the widest and darkest of the three. Sometimes, however, the two bands at E and F are of equal intensity, while that at D is always comparatively faint, and often imperceptible.

When the biliary salts, used for Pettenkofer's test, are dissolved in alcohol, as in the foregoing experiments, the color of the fluid produced is less pure and bright than when water is employed as the solvent. There is also more opacity, in proportion to the amount of color present; and, consequently, the spectrum is comparatively dim, and the absorption-bands less distinct than with a watery solution.

But when Pettenkofer's test is applied to a watery solution of the biliary salts sufficiently concentrated to give a distinct violet or purple color, the fluid produced is too opaque for spectroscopic examination, and consequently requires to be diluted. If water, however, be added to it, there is immediately formed a whitish turbidity, and the purple color disappears. This difficulty does not present itself with an alcoholic solution, which may be diluted to any degree by the addition of alcohol, without exhibiting any turbidity or losing its color. It was for this reason that alcohol was employed as the solvent in the experiments of Dr. Schenk.

It is not necessary, however, to use alcohol for this purpose.

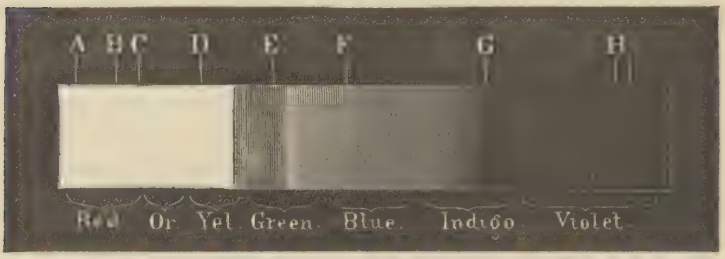
If we dilute the watery solution after treatment by Pettenkofer's test, it is true that this produces a turbidity and decolorization, as above described; but the turbidity can be easily cleared up again, and the color restored, by a further addition of sulphuric acid. This method, which was suggested by one of my assistants, Mr. Geo. A. Spalding, I have always found to be perfectly successful. In this way the watery solution may be diluted at pleasure, and its color and transparency restored without difficulty.

It is not even indispensable to resort to this treatment, in order to secure a clear and transparent fluid for spectroscopic examination, provided the solution be made at first sufficiently dilute. If pure sodium glycocholate be dissolved in water in the proportion of one part to 500, and the solution treated with Pettenkofer's test, it gives in a few moments a clear violet-pink color, which afterward becomes a rich purple. This fluid is so opaque that, when placed before the slit of the spectroscope in a layer of one centimetre, it extinguishes completely every thing but the red; and yet it may be diluted with water without showing any turbidity or losing its color. A watery solution of this strength is, therefore, amply sufficient to exhibit the reaction of Pettenkofer's test, and the spectroscopic appearances belonging to it; and it does not require to be corrected, after dilution, by the addition of sulphuric acid.

In many repeated observations, I have found that it makes a very decided difference in the spectrum of Pettenkofer's test whether alcohol or water be used as the solvent fluid. With the alcoholic solution there are two bands, at E and F, as already described. With the watery solution, there is only one. If the pure sodium salts of either glycocholic or taurocholic acid, in watery solution, be treated with Pettenkofer's test, the spectrum shows only the absorption-band at E. This band, in a fluid of the requisite degree of strength, is very dark and tolerably well defined, extending usually from D 50 E to E 25 F. Its limits are of course farther extended by increasing the thickness of the layer of the fluid under observation. Beyond the band, the spectrum is comparatively dim, and ends gradually toward the situation of the line G.

A similar appearance is produced if the biliary salts of dried ox-bile be crystallized by extraction with alcohol and precipitation with ether. The same biliary salts, obtained in this way, if dissolved in alcohol, will give a spectrum with the two bands at E and F, and, if dissolved in water, will give only the band at E.

Fig. 4.



Spectrum of Pettenkofer's Test with the Biliary Salts in Watery Solution.

In some instances I have found that the violet-pink fluid, which at first gave only the band at E, became, after standing for twenty-four hours, somewhat turbid and dingy in color, and then showed the two absorption-bands, both rather indistinctly. But, on being again treated with sulphuric acid, it resumed its former transparancy and color, and at the same time again showed only the band at E, which was perfectly distinct; the other having disappeared. A watery solution, also, which shows only the E band, if diluted with alcohol instead of water, will at once give indication, more or less distinct, of the band at F. I cannot help considering, therefore, the band at E as the only characteristic feature in the spectrum of a watery solution of the biliary salts, when treated with Pettenkofer's test.

My own observations agree with those of Dr. Schenk as to there being no difference in the spectrum produced, whether we use the salts of glycocholic or of taurocholic acid. In point of fact, the first step in applying Pettenkofer's test, that is, the addition of sulphuric acid, converts both these substances into cholic acid, and the purple color which finally appears is really formed in each case by cholic acid, which may thus be derived from either or both of the original biliary salts.

The pink and purplish-red fluid, produced by Pettenkofer's test with both codeine and morphine, has a spectrum very similar to that of the biliary salts. If the ruddy color of the fluid be strongly pronounced, its spectrum, even when viewed in a layer of one centimetre, is very short, terminating completely about midway between D and E, or even before that point, showing the red and yellow clear and bright, but very little of the green. If diluted with water, the mixture is not rendered turbid, but its color is very much reduced, being soon changed to a faint amber or often to a light apple-green, while the former peculiarities of the spectrum disappear. The best way is to place the fluid before the slit of the spectroscope in a layer of two centimetres before its color is fully developed, and while it is still of a light pink. The color then gradually becomes more pronounced, and, when it has attained the proper degree of strength, the spectrum exhibits a certain though ill-defined absorption-band at E, extending from about D 50 E to E 25 F. Beyond the band the whole spectrum is very dim, and terminates gradually between F and G.

The distinction between the spectrum of Pettenkofer's test with biliary salts and that with the opium alkaloids is, that in the former case the absorption-band at E is very marked and distinct, and often quite black, when viewed in a layer of two centimetres' thickness, while in the latter it is always dim and very ill-defined. With the biliary salts also, the fluid may frequently be diluted with its own or even twice its volume of water, and the absorption-band still remain plainly visible; but with morphine or codeine a very moderate dilution rapidly destroys the character of the spectrum and causes the absorption-band to disappear.

The violet-colored fluid produced by Pettenkofer's test with albumen has a well-marked and peculiar spectrum, easily distinguishable from that belonging to the biliary salts. If tolerably dense, it requires to be diluted with water for spectroscopic examination, and afterward cleared up by the further addition of sulphuric acid. The spectrum then shows a single absorption-band, extending from somewhere about the line E to the line F, and occupying the intermediate space. In concentrated specimens it may begin so far toward the left

as D 65 E and extend thence to F. If the albuminous liquid be more dilute, it may reach only from E 24 F to F. It is therefore always limited on the right by the line F, but extends farther and farther toward E and D, according to the degree of the concentration of the liquid. Its edges are not very well defined, but are more distinct when the band is narrow than when it is wide. Beyond the band the refrangible portion of the spectrum is quite dim.

FIG. 5.

SPECTRUM OF PETTENKOFER'S TEST WITH ALBUMEN.

Finally, it would be desirable to know in what degree Pettenkofer's test is reliable for detecting small quantities of the biliary salts, and whether spectroscopic examination be capable of increasing the delicacy of the test.

Merck's pure sodium glycocholate, dissolved in water in the proportion of one part to fifty, and treated with Pettenkofer's test, makes at once a fluid of a clear violet-pink color, passing rapidly into a strong ruby red, the spectrum of which shows the absorption-band at E excessively wide and black, when viewed in a thickness of one centimetre.

The same substance, dissolved in 500 parts of water, makes, in a few moments after application of the test, a clear violet-pink fluid, in which the absorption-band at E is distinct though not very strong; but after standing for an hour it is of too opaque a purple to be examined without dilution, and when properly reduced shows the E band distinctly.

Dissolved in 1,000 parts of water, it readily yields a clear violet-pink fluid which, in a layer of one centimetre, shows the E band with perfect distinctness.

Dissolved in 2,000 parts of water, it changes color very slowly after the application of the test, but at the end of fif-

teen minutes becomes a clear violet-pink, and shows the E band distinctly in layers of one centimetre.

Dissolved in 3,000 parts of water and treated with Pettenkofer's test, it acquires slowly a very dilute, clear, pinkish-amber tinge, and shows only faint indication of the E band in a layer of two centimetres' thickness. After standing for an hour, it has a distinct cherry-red color, with a tinge of violet; and in this condition, if viewed in a layer of two centimetres, the E band is plainly visible, but too faint and ill-defined to be serviceable as a test.

With the sodium taurocholate the sensibility of Pettenkofer's test is greater still. If this salt be dissolved in 1,000 parts of water and the test applied, it produces immediately a light violet-pink fluid, in which the E band is distinctly visible; but after standing for an hour it is of a fine clear violet, and the E band is very strong and black, in layers of one centimetre.

If dissolved in 3,000 parts of water it assumes, in fifteen minutes after the application of the test, a light, clear cherry-red color, which afterward has a tinge of violet, and, in a layer of two centimetres, shows the E band distinctly.

In higher degrees of dilution it fails to respond to the test. If dissolved in 5,000 parts of water, the fluid assumes slowly an almost imperceptible rosy tint, which is not altered even after standing for six hours, and shows no visible absorption-band in a layer of two centimetres' thickness.

With both the glycocholate and taurocholate, if dissolved in alcohol, the reaction of Pettenkofer's test is perceptibly less delicate than when employed in a watery solution. The colors produced are less clear and the absorption-bands less distinct.

Sodium glycocholate, therefore, may be detected by Pettenkofer's test, in a watery solution of one part to two thousand, and the taurocholate in a solution of one part to three thousand, if the test be applied with care. The spectroscopic examination of the fluid is useful in identifying the reaction as due to the presence of the biliary salts, but it does not perceptibly increase the delicacy of the test. The characteristic absorption-bands are more or less marked, according

to the strength and purity of the red or violet color to the eye; and at every successive degree of dilution they become faint and ill-defined, exactly in proportion as the fluid itself loses its distinctive coloration. I do not think we could ever detect the presence of the biliary salts by spectroscopic examination, in a fluid which did not show any distinct ruby or violet color.

The results of the preceding observations may be stated as follows:

I. The spectrum of bile is characterized, as a general rule, by an absorption-band at C.

II. The existence and intensity of this band are proportional to the predominance of green in the color of the bile.

III. The spectrum of the bile is also distinguished by a diminution or absence of the orange and yellow, and a corresponding extension of the red and green.

IV. There are sometimes also two other absorption-bands, comparatively uncertain and ill-defined, at D and at D 30 E.

V. The pure biliary salts in alcoholic solution, treated by Pettenkofer's test, give a spectrum with absorption-bands at E and F.

VI. In a watery solution, treated by the same test, they give a spectrum with but one absorption-band, namely, at E.

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